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(54) Title: LOW DOSAGE OF INTERFERON TO ENHANCE VACCINE EFFICIENCY

(57) Abstract

A biologically active interferon can be administered to an animal in conjunction with the administration of a vaccine to improve the vaccine efficiency and allow the use of a smaller vaccination dose. This procedure will cause a less severe vaccine infection in the animal than if no interferon was administered.

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AMENDED CLAIMS

[received by the International Bureau on 7 July 1987 (07.07.87); original claims 1-13 unchanged; 14-17 amended; 18-22 new (2 pages)]

14. A combination for vaccinating warm-blooded vertebrates, including:

a suspension of attenuated or killed microorganisms suitable for inducing immunity to an infectious disease; and

at least one dose of a biologically active interferon in a form for administration in an amount no greater than about 5 IU of interferon/lb (11 IU/kg) of body weight;

in which combination the suspension and the dose of interferon are optionally in a form for independent administration.

15. The combination of claim 14 where the suspension of microorganisms is in a form for oral administration and includes the dosage of interferon.

16. The combination of claim 14, where the interferon is in a dosage form for oral administration.

17. The combination of claim 14, where the interferon is human alpha interferon.

18. A method for manufacturing a composition for use in a method of enhancing vaccine efficiency in warm-blooded vertebrates, said method of manufacture characterized by combining interferon and a pharmaceutically acceptable carrier for oral administration of said interferon in an amount no greater than about 5 IU/lb (11 IU/kg) of body weight.

19. The method of claim 18 wherein the interferon is alpha-interferon.

20. A vaccine efficiency enhancing composition containing interferon for oral administration to a warm-blooded vertebrate in conjunction with vaccination

of said vertebrate, said composition including interferon at a unit dosage amount no greater than about 5 IU/lb (11 IU/kg) of body weight and a pharmaceutically acceptable carrier therefor.

21. The composition of claim 20 wherein the unit dosage amount of interferon is about 0.01 to about 1.0 IU/lb (about 0.022 to about 2.2 IU/kg) of body weight.

22. The composition of claim 20 wherein the interferon is alpha interferon.

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(54) Title: LOW DOSAGE OF INTERFERON TO ENHANCE VACCINE EFFICIENCY

(57) Abstract

A biologically active interferon can be administered to an animal in conjunction with the administration of a vaccine to improve the vaccine efficiency and allow the use of a smaller vaccination dose. This procedure will cause a less severe vaccine infection in the animal than if no interferon was administered.

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LOW DOSAGE OF INTERFERON TO ENHANCE VACCINE EFFICIENCY

This invention relates generally to methods of enhancing the efficiency of vaccines in warm-blooded vertebrates. The methods involve administering interferon to a warm-blooded vertebrate in conjunction with administration of a vaccine.

"Interferon" is a term generically comprehending a group of vertebrate glycoproteins and proteins which are known to have various biological activities, such as antiviral, antiproliferative, and immunomodulatory activity in the species of animal from which such substances are derived. The following definition for interferon has been accepted by an international committee assembled to devise a system for the orderly nomenclature of interferons: "To qualify as an interferon a factor must be a protein which exerts virus nonspecific, antiviral activity at least in homologous cells through cellular metabolic processes involving synthesis of both RNA and protein." Journal of Interferon Research, 1, pp. vi (1980).

Since the first descriptions of interferon by Isaacs and Lindeman [See, Proc. Roy. Soc. London (Ser. B), Vol. 147, pp. 258 et seq. (1957) and U.S. Patent No. 3,699,222], interferon has been the subject of intensive research on a worldwide basis. Publications abound concerning the synthesis of interferon; M. Wilkinson and A. G. Morris, Interferon and the Immune System 1: Induction of Interferon by Stimulation of the Immune System, Interferons: From Molecular Biology to Clinical Application, Eds: D. C. Burke and A. G. Morris, Cambridge Univ. Press, 1983, pp. 149-179; P. I. Marcus, Chapter 10, Interferon Induction by Virus, Interferons and Their Applications, Eds: P. E. Came and W. A. Carter, Springer Verlag, (Handbook of Experimental Pharmacology V. 71) 1984, pp. 205-232; its proposed molecular characterizations; P. B. Sehgal, How Many Human Interferons Are There? Interferon 1982, Ed: I. Gresser, Academic Press, 1982, pp. 1-22; J. Collins, Structure and Expression of the Human Interferon Genes, Interferons: From Molecular Biology to Clinical Application, Eds: D. C. Burke and A. G. Morris, Cambridge Univ. Press, 1983, pp. 35-65; K. C. Zoon and R. Wetzel, Chapter 5, Comparative Structures of Mammalian Interferons, la: Interferons and Their Applications, Eds: P. E. Came and W. A. Carter, Springer Verlag, (Handbook of Experimental Pharmacology V. 71) 1984, pp. 79-100; its clinical applications; M. Krim, Chapter 1, Interferons and Their Applications: Past, Present, and Future, Interferons and Their Applications, Eds: P. E. Came and W. A. Carter, Springer Verlag, (Handbook of Experimental Pharmacology V. 71) 1984; S. B. Greenberg and M. W. Harmon, Chapter 21, Clinical Use of Interferons: Localized Applications in Viral Diseases, Ibid. pp. 433-453; and proposed mechanisms of its antitumor, antiviral, and immune system activities. G. M. Scott, The Antiviral Effects of Interferon, From Molecular Biology to Clinical Application, Eds: D. C. Burke and A.

G. Morris, Cambridge Univ. Press, 1983, pp. 279-311; M. McMahon and I. M. Kerr, The Biochemistry of the Antiviral State, Ibid. pp. 89-108; J.S. Malpas, The Antitumor Effects of Interferon, Ibid. pp. 313-327; J. Taylor-Papadimitrion, The Effects of Interferon on the Growth and Function of Normal and Malignant Cells, Ibid. pp. 109-147.

Because of the intensity and disparate origins of research concerning interferon and its characteristics and uses, there exists a substantial lack of uniformity in such matters as classification of interferon types. There are also numerous, sometimes contradictory, theories concerning the mode of action of interferon in producing clinical effects. The following brief summary of the current state of knowledge regarding interferon will aid in understanding the present invention.

Although originally isolated from cells of avian origin (chick allantoic cells), interferon production has been observed in cells of all classes of vertebrates, including mammals, amphibians, and reptiles. Interferon production by vertebrate cells is seldom spontaneous but is often readily "induced" by treatment of cells (in vivo or in vitro) with a variety of substances including viruses, nucleic acids (including those of viral origin as well as synthetic polynucleotides), lipopolysaccharides, and various antigens and mitogens.

Interferons have generally been named in terms of the species of animal cells producing the substance (e.g., human, murine, or bovine), the type of cell involved (e.g., leukocyte, lymphoblastoid, fibroblast) and, occasionally, the type of inducing material responsible for interferon production (e.g., virus, immune). Interferon has been loosely classified by some researchers according to induction mode as either Type I or Type II, with the

former classification comprehending viral and nucleic acid induced interferon and the latter class including the material produced as a lymphokine through induction by antigens and mitogens. More recently, the international committee devising an orderly nomenclature system for interferon has classified interferon into types on the basis of antigenic specificities. In this newer classification, the designations alpha (α), beta (β), and gamma (γ) have been used to correspond to previous designations of leukocyte, fibroblast, and type II (immune) interferons, respectively. Alpha and beta interferons are usually acid-stable and correspond to what have been called type I interferons; gamma interferons are usually acid-labile and correspond to what has been called type II interferons. The international committee's nomenclature recommendations apply only to human and murine interferons. Journal of Interferon Research, 1 pp. vi (1980).

Determination of precise molecular structures for interferon was for some time beyond the capacities of the art. In the years since interferon was first characterized as proteinaceous on grounds of its inactivation by trypsin, attempts to purify and uniquely characterize were frustrated by its high specific activity as well as its apparent heterogeneity. Presently, some precision in determining molecular structure has been achieved for interferon. See P. B. Sehgal, supra; J. Collins, supra; and K. C. Zoon and R. Wetzel, supra.

In its earliest applications, interferon was employed exclusively as an antiviral agent and the most successful clinical therapeutic applications to date have been in the treatment of viral or virus-related disease states. It became apparent, however, that exogenous interferon was sometimes capable of effecting regression or remission of

various metastatic diseases. An overview of current clinical trials of interferon as an antiviral and antiproliferative therapeutic agent through early 1983 is contained in The Biology of the Interferon System 1983, Proceedings of the Second International TNO Meeting on the Biology of the Interferon System, Rotterdam, The Netherlands, 18-22 April 1983, and Antiviral Research, March 1983, Special Abstract Issue, Elsevier/North-Holland Biomedical Press, Netherlands.

The clinical agent of choice in this work has been human leukocyte interferon, "mass-produced" by procedures involving collection and purification of vast quantities of human buffy coat leukocytes, induction with virus, and isolation from culture media. The need for interferon of human source is, of course, consistent with the long-standing conclusion that interferon is "species specific", i.e., biologically active, in vivo, only in species homologous to the source cells.

In the work described above, interferon has been administered parenterally, i.e., intramuscularly and intradermally, with some successful topical and intranasal usages having been reported. It has seldom been administered intravenously because of substantial adverse effects attributable to "contaminants" in crude and even highly purified isolates. The invention of applicant described in U.S. Patent No. 4,462,985, and in PCT International Application No. PCT/US 81/01103, filed August 18, 1981, published March 4, 1982, concerns the use of interferon of heterologous species origin, and also involves oral administration of interferon. Prior to these disclosures, there had been no reports of therapeutically successful oral administration of interferon. This circumstance was consistent with the widely held belief that interferon

would not withstand exposure to a digestive environment such as that found in mammals.

In addition to use in antiviral and antitumor therapy, interferon has rather recently been noted to possess immunomodulatory effects, both immunopotentiating and immunosuppressive in nature. B. Lebleu and J. Content, Mechanisms of Interferon Action: Biochemical and Genetic Approaches, Interferon 1982, Ed: I. Gresser, Academic Press, 1982, pp. 47-94; M. Moore, Interferon and the Immune System, 2: Effect of IFN on the Immune System, Interferons: From Molecular Biology to Clinical Application, Eds: D. C. Burke and A. G. Morris, Cambridge Univ. Press, 1983, pp. 181-209; H. Smith-Johannsen, Y-T Hou, X-T Liu, and Y-H Tan, Chapter 6, Regulatory Control of Interferon Synthesis and Action, Interferons and Their Applications, Eds: P. E. Came and W. A. Carter, Springer Verlag, (Handbook of Experimental Pharmacology V. 71) 1984, pp. 101-135; J. L. Raylor, J. L. Sabram, and S. E. Grossberg, Chapter 9, The Cellular Effects of Interferon, Ibid. pp. 169-204; J. M. Zarling, Effects of Interferon and Its Inducers on Leukocytes and Their Immunologic Functions, Ibid. pp. 403-431; R. Ravel, The Interferon System in Man: Nature of the Interferon Molecules and Mode of Action, Antiviral Drugs and Interferon: The Molecular Basics of Their Activity, Ed: Y. Becker, Martinus Nijhoff Pub., 1984, pp. 357-433.

Further, "new" biological activities for exogenous and endogenous interferon are consistently being ascertained. K. Berg, M. Hokland, and I. Heron, Biological Activities of Pure HuIFN-Alpha Species, Interferon, Properties, Mode of Action, Production, Clinical Application, Eds: K. Munk and H. Kirchner, (Beitrage zur Onkologie V. 11) pp. 118-126; S. Pestka et al, The Specific Molecular Activities of Interferons Differ for

Antiviral, Antiproliferative and Natural Killer Cell Activities, The Biology of the Interferon System, 1983, Eds: E. DeMaeyer and H. Schellekens, pp. 535-549; P. K. Weck and P. E. Cane, Chapter 16, Comparative Biologic Activities of Human Interferons, Interferons and Their Applications, Eds: P. E. Came and W. A. Carter, Springer Verlag, (Handbook of Experimental Pharmacology V. 71) 1984, pp. 339-355.

One infectious disease which has not been controlled, by interferon or other means, is bovine respiratory disease complex (BRDC). BRDC is an all-encompassing term describing an acute, contagious infection of cattle characterized by inflammation of the upper respiratory passages and trachea. BRDC leads to pneumonia with clinical signs of dyspnea, anorexia, fever, depression, mucopurulent nasal discharge and mucopurulent ocular discharge, all of which result in high morbidity and mortality. BRDC is a major cause of disease loss in beef cattle. The economic loss to cattlemen for treatment, weight loss, death loss, and culling is estimated to be $333,000,000 annually (National Cattlemen's Association, 1980).

When BRDC symptomology is observed in cattle after transport to feedlots or pastures, it is commonly called "shipping fever." On their way to the feedlot, calves are subjected to the stresses of intensive management techniques, transportation without food or water, and a variety of infectious agents. Upon arrival at the feedlot, processing exposes the calves to the additional stresses of weaning, castration, dehorning, branding, eartagging, worming, vaccination, and delousing. In many situations, calves are stressed still further by changes in diet and environmental factors.

The infectious agents to which calves entering the marketing system are exposed include viruses (infectious bovine rhinotracheitis (IBR), non-IBR herpesviruses, parainfluenza type 3 (PI3), bovine viral diarrhea (BVD), respiratory syncytial, adenoviruses, enteroviruses, rhinoviruses, parvoviruses, and reoviruses), bacteria (Pasteurella hemolytica, Pasteurella multocida, and Hemophilus somnus), mycoplasma (M. dispar, M. bovirhinis, M. bovis, and M. arginini), and Chlamydia.

The IBR, BVD, and PI3 viruses are three of the infectious agents that are most commonly isolated by veterinary diagnostic laboratories in cases of BRDC. While some commercial vaccines for IBR, BVD, and PI3 are available, they have not been completely satisfactory in the past, partly because vaccination of calves stressed by shipping can exacerbate the clinical signs of the disease. Also, some calves will not develop antibodies after vaccination, leaving them still susceptible to infection. Furthermore, many commercial vaccines are designed to provide protection no sooner than 14 days after vaccination, tracking the U.S. Department of Agriculture, Bureau of Biologics, immunogenicity test. Because of the imperfections of the vaccination treatments used in the past and the enormous economic losses involved, a need exists for improved methods of preventing and treating bovine respiratory disease.

In a more general sense, a need exists for improved methods of vaccinating cattle and other warm-blooded vertebrates. Present vaccines are sometimes harmful. For example, they can produce a detrimental vaccine infection. If the efficiency of vaccines could be improved, then the amount of killed or attenuated microorganisms needed to give an effective vaccination dose could possibly be reduced. This would in turn decrease the chances of a

detrimental vaccine infection and reduce the cost of the vaccine. The possibility of producing a quicker antibody response to vaccination would also exist.

Applicant has made the surprising discovery that administration of a biologically active interferon in conjunction with the administration of a vaccine can enhance the vaccine's efficiency.

A method in accordance with the present invention of enhancing the efficiency of a vaccine in warm-blooded vertebrates includes or comprises administering to a warm-blooded vertebrate, in conjunction with the administration of a vaccine, a biologically active interferon in a dosage no greater than about 5 IU/lb of body weight per day. The presently preferred dosage is about 1.0 IU of human interferon alpha per pound of body weight per day.

The interferon can be administered to the animal through a number of routes, such as orally, intranasally, intramuscularly, or intravenously. Oral administration is presently preferred. It can be administered in a single dose, either simultaneously with the administration of the vaccine or within about one day before or after the vaccine administration. Alternatively, the interferon can be administered in several doses, for example, by administering a dose on two or more of the days in the period consisting of the day before vaccine administration, the day of vaccine administration, and the day after vaccine administration. If the interferon and vaccine are administered simultaneously, they can be administered separately or mixed together.

The advantages of the present invention include enhanced vaccine efficiency by promotion of antibody production, earlier antibody production, and reduced

vaccine costs as a result of using a smaller amount of microorganisms to produce an effective dose. As an example of the latter advantage, current IBR vaccine dosages are about $10^{5.5}$ to $10^{6.0}$ TCD_{50}/ml. Applicant believes that the present invention should allow reduction of this dosage by a factor of about ten to one hundred.

The present invention achieves its effects with low doses of interferon. In addition to the favorable biological activity, using small doses naturally makes these methods less expensive than if they used large doses. Methods in accordance with the present invention are applicable to animal species such as bovine, porcine, caprine, ovine, avian, feline, canine, and equine species, as well as humans.

The interferon administered can be of heterologous or homologous species origin. ("Heterologous species origin" means that the interferon has been derived from cells of a species other than that to which it is administered.)

The optimum dosage of interferon varies somewhat species to species, and probably animal to animal. Also, effects similar to those produced by a given daily dosage administered for a given number of days might be achieved by administering a slightly lower dosage for a slightly greater number of days, or a slightly higher dosage for a slightly smaller number of days. Along the same lines, if an animal has an infection that is causing it to secrete some interferon naturally, the dosage to be administered might be reduced somewhat to achieve the same biological effects.

Applicant has filed several previous patent applications relating to methods of using interferon (U.S. Patents 4,462,985 and 4,497,795, and U.S. patent appli-

cation serial no. 688,868, filed on January 4, 1985). They are incorporated in this specification by reference.

The methods of the present invention can use interferons produced by methods known to those skilled in the art. One specific suitable method of preparing an interferon is described below in Example 1. Examples 2-6 illustrate methods of using interferon. All geometric mean titers of antibody are expressed to the base 2 in the following examples.

EXAMPLE 1

Human interferon alpha can be prepared through the following procedure, commonly referred to as the Cantell procedure. The process begins with packs of human leukocytes, obtained in this case from the Gulf Coast Regional Blood Center, Houston, Texas. The buffy coats in these packs are pooled into centrifuge bottles, and then are diluted with 0.83% ammonium chloride. The mixture is incubated for 15 minutes with intermittent shaking, and is then centrifuged for 20 minutes at 2000 rpm. The supernatant is discarded, and the cell pellets are resuspended with a minimal volume of sterile phosphate buffered saline (PBS). The mixture is then diluted with ammonium chloride and centrifuged. The supernatant is again discarded, and the remaining cell pellets are resuspended with a minimal volume of a tissue culture medium such as Minimal Essential Medium (MEM), available from KC Biological. The cell concentration is determined with a Coulter counter.

Interferon induction takes place in glass or plastic bottles. The induction medium contains MEM, 75mM Hepes (available from Calbiochem), 75mM Tricine (available from Sigma Chemical Co.), human agamma serum (18mg/ml), and gentamycin sulfate (from M.A. Bioproducts; 50mcg/ml). The

cells are added to the induction vessels at a final concentration of about 5 to 10 million cells per milliliter. The induction vessel is incubated in a 37°C water bath, and interferon alpha is added as a primer.

After two hours, Sendai virus is added to the induction mixture. This causes alpha interferon to be produced in the supernatant by the leukocytes. After a 12-18 hour incubation time, the induction mixture is centrifuged. The cells are discarded, and the supernatant is then purified.

The crude interferon is chilled to 10°C or below in an ice bath. Five molar potassium thiocyanate is added to obtain a final concentration of 0.5M. This solution is stirred for 15 minutes, and then its pH is lowered to 3.3 by adding hydrochloric acid. The mixture is then centrifuged at 2800 rpm for 30 minutes, and the supernatant is discarded.

The pellets are then resuspended in 95% ethanol and are stirred for 15 minutes. This suspension is centrifuged at 2800 rpm for 20 minutes, and the pellets are discarded. The pH of the supernatant is then adjusted to 5.8 with sodium hydroxide. The mixture is stirred for 10 minutes, and then centrifuged at 2800 rpm for 20 minutes. The pellets are discarded. The pH of the supernatant is then adjusted to 8 with sodium hydroxide. This solution is stirred for 10 minutes, followed by centrifugation at 2800 rpm for 20 minutes. The supernatant is discarded, and the pellets are resuspended with 0.5M potassium thiocyanate in a 0.1M sodium phosphate buffer. This suspension is stirred at 4°C.

Next, the suspension is centrifuged at 2800 rpm for 20 minutes, and the pellets are discarded. The pH of the

supernatant is adjusted to 5.3 with hydrochloric acid. After stirring for 10 minutes and centrifugation, the pH of the supernatant is adjusted to 2.8 with hydrochloric acid, followed by further stirring for 20 minutes. This mixture is centrifuged at 2800 rpm, and the resulting pellet is purified human interferon alpha.

The pellet is resuspended with 0.5M potassium thiocyanate in 0.1M sodium phosphate buffer, having a pH of 8.0. It is then dialyzed against PBS at 4°C, with two changes of PBS. This mixture is then centrifuged and the precipitate is discarded. The remaining purified alpha interferon is sterilized by filtration through a 0.2 micron filter.

A human interferon alpha is produced in accordance with this procedure by Immuno Modulators Laboratories, Inc., Stafford, Texas, and sold under the trademark Agriferon®-C for use in cattle.

Other procedures known to those skilled in the art are available for making interferons, such as human interferon alpha and human interferon gamma. For example, U.S. Patents 4,376,821 and 4,460,685 disclose methods of making human interferon gamma. A method of making bovine fibroblast interferon is disclosed in applicant's U.S. patent 4,462,985.

EXAMPLE 2

Forty feeder calves were randomly assigned to four treatment groups of ten calves each. All of the calves were initially seronegative to IBR virus. The calves were given either a placebo or human interferon alpha orally in three consecutive daily dosages of 0.05, 0.5, or 5.0 IU/lb body weight, respectively. A dose of interferon or the

placebo was given on the day before, the day after, and the day of IBR virus inoculation. Each calf was given 10^3 plaque forming units (PFU) of IBR virus per nostril.

Tables 1-3 show the results of this test.

TABLE 1

Number of Calves with a Temperature of at Least 104°F

Treatment Group	Days After IBR Virus Inoculation														
	-1	0	1	2-4	5	6	7	8	9	10	14	18	19	23	25	Total
Control	1	0	2	1	0	1	1	2	0	0	3	4	1	2	0	18
0.05 IU/lb	1	1	1	0	2	3	4	4	3	4	2	2	0	2	0	29
0.5 IU/lb	0	0	1	0	2	5	7	6	3	1	0	0	0	0	0	25
5.0 IU/lb	1	1	1	1	3	2	6	4	1	0	1	2	0	2	0	25

TABLE 2

Geometric Mean Serum Antibody Titers to IBR Virus

Treatment Group	Days After Virus 0	14	25
Control	0	1.9	29.8
0.05 IU/lb	0	3.7	27.9
0.5 IU/lb	0	8.8	24.3
5.0 IU/lb	0	4.6	21.1

TABLE 3

Geometric Mean Titers of Plaque Forming Units (PFU) of IBR Virus Excretion

	Days after Inoculation				
Treatment Group	0	3	7	10	14
Control	0	2	204	6,310	12,078
0.05 IU/lb	0	21	3,396	174,582	298
0.5 IU/lb	0	221	20,749	20,184	7
5.0 IU/lb	0	71	4,130	43,451	132

As Table 1 shows, the rectal temperatures of the cattle differ significantly among the four treatment groups after inoculation. More calves given the 0.5 IU/lb dosage than controls had a fever of at least 104°F at 5, 6, 7, 8 and 9 days after inoculation. More control calves had a fever greater than 104°F at 14 and 18 days after virus inoculation.

Antibodies to IBR virus were produced in all groups. However, Table 2 shows that antibody production occurred significantly faster in the group treated with 0.5 IU/lb. Nasal excretion of IBR virus also occurred and disappeared sooner in the 0.5 IU/lb treatment group, as shown in Table 3. Significantly more virus was excreted by the 0.5 IU/lb treatment group than by controls at 3 and 7 days after IBR virus inoculation, but significantly less virus was excreted at 14 days after inoculation. At 14 days, only 7 PFU of virus were excreted by calves given 0.5 IU/lb compared to over 12,000 PFU of virus excretion from controls.

In summary, human interferon alpha administered orally at 0.5 IU/lb of body weight significantly stimulated antibody development at 14 days after IBR virus inoculation and significantly reduced IBR virus shedding at 14 days after inoculation.

EXAMPLE 3

A number of light weight feeder calves (average weight 460 lbs)were shipped to a feedlot, and subsequently many experienced a natural shipping fever outbreak. The calves were not vaccinated. The calves were tested for the presence of antibody to PI3 virus. The calves that tested seronegative were divided into three treatment groups, and human interferon alpha or placebo was administered to the three groups in three consecutive daily oral doses of 0, 0.1, or 1.0 IU/lb of body weight, respectively. Table 4 shows the results of this test.

TABLE 4

Serology to Parainfluenza-3 Virus
28 Days after Arrival at Feedlot

No. of Seronegative Calves	Treatment Group (IU/lb body wt.)	Seroconversion at 28 Days
31	0.00	71%
30	0.10	96%
33	1.00	75%

As Table 4 shows, the calves treated with the 0.1 IU/lb dose achieved significantly better seroconversion to PI3 virus during natural disease than calves treated with 1.0 IU/lb or with placebo.

EXAMPLE 4

A number of calves were divided into six treatment groups of eighteen each. Two of the treatment groups were given a full dose of vaccine, two groups were given a hundred fold reduced dose of vaccine, and the remaining two groups were not vaccinated. For each of the pairs of treatment groups, one group was treated orally with interferon and one was not.

The vaccine was an IBR-PI3-BVD modified live virus vaccine obtained from CEVA Labs, serial no. 71020L39, and contained at least $10^{5.7}$ TCD_{50}/ml of IBR virus, $10^{4.3}$ TCD_{50}/ml of BVD virus, and $10^{4.7}$ TCD_{50}/ml of PI3 virus. The vacinnation was administered intramuscularly. The interferon treatment was a single oral dose of human interferon alpha at the rate of 1.0 IU/lb of body weight

and was administered at the time of vaccination. Tables 5-7 show the results of this test.

TABLE 5

Geometric Mean Antibody Titers to IBR Virus 13 and 25 Days after Vaccination of Seronegative Calves

Vaccine Dose	Treatment	No. of Calves	GMT after Vaccination on Day 13	GMT after Vaccination on Day 25
1:100X	Placebo	14	1.8	5.7
1:100X	Interferon	15	2.3	4.2
1X	Placebo	15	2.9	5.3
1X	Interferon	16	3.0	7.3

TABLE 6

Geometric Mean Antibody Titers to BVD Virus 13 and 25 Days after Vaccination of Seronegative Calves

Vaccine Dose	Treatment	No. of Calves	GMT after Vaccination on Day 13	GMT after Vaccination on Day 25
1:100X	Placebo	15	1.3	5.5
1:100X	Interferon	11	1.4	3.1
1X	Placebo	14	1.3	11.2
1X	Interferon	15	2.4	12.7

TABLE 7

Seroconversion to PI3 Virus
0 and 25 Days after Inoculation
with a Full Dose of Vaccine

		PI3 Virus Antibody Titer on Day	
Calf	Treatment	0	25
A	Placebo	<4	<4
B	Placebo	<4	<4
C	Interferon	<4	16
D	Interferon	<4	8
E	Interferon	<4	4
F	Interferon	<4	8
G	Interferon	<4	4

Table 5 shows that calves treated with interferon produced slightly higher geometric mean titers to IBR virus at 13 days after vaccination than did calves not treated with interferon at equal vaccine dosages. Table 6 shows that the same was generally true for geometric mean antibody titer to BVD virus. Table 7 shows a significant improvement in seroconversion in calves treated with interferon versus controls. Neither of the two calves (A and B) reported in this table who were not treated with interferon achieved a PI3 virus antibody titer of as high as four on the 25th day after vaccination. However, each of the five calves (C-G) treated with interferon had achieved a titer of at least four by that time.

EXAMPLE 5

One hundred calves were divided into five treatment groups of twenty each. One group was used as controls and was vaccinated but not treated with interferon. The other

four groups were vaccinated and treated with either one dose of lyophilized interferon, two doses of lyophilized interferon, one dose of interferon that had been frozen, or two doses of interferon that had been frozen. The interferon was human interferon alpha and each oral dose was 1.0 IU/lb of body weight. The vaccination was with the same vaccine identified in Example 4. Tables 8-10 show the results of this test.

TABLE 8

Geometric Mean Titers of Antibody
of Seronegative Calves
to IBR Virus 14 Days after Vaccination

		14 Days after Vaccination	
Treatment	No. of Calves	No. of Calves With Titer > 8	GMT
Control	16	7	4.2
lyo. once	18	8	4.0
lyo. twice	18	11	5.0

TABLE 9

Geometric Mean Titers of Antibody of Seronegative Calves to BVD Virus 28 Days After Vaccination

Treatment	No. of Calves	GMT
Control	15	12.7
lyo. once	17	14.7
lyo. twice	12	13.5
froz. once	19	19.2
froz. twice	16	15.3

TABLE 10

Fever and Sickness

		Fever		Antibiotic
No. of Calves	Treatment	Peak (°F)	Duration > 104°F	Treatment Days
16	Control	105.4	2.88	6.4
18	lyo. once	105.5	2.28	5.8
17	lyo. twice	105.5	2.35	5.9
16	froz. once	105.1	2.31	5.6
18	froz. twice	105.3	3.11	6.7

Table 8 shows that a slightly higher percentage of calves achieved a geometric mean antibody titer to IBR virus of at least eight by the fourteenth day after vaccination if they had been treated with interferon. Table 9 shows that all interferon treatment groups produced higher GMT's of antibody to BVD virus at 28 days

than did the controls. Table 10 shows that most groups of calves treated with interferon had a shorter fever duration.

EXAMPLE 6

One hundred two light weight feeder steers and bulls (average pay weight 442 lbs.) were purchased from an order buyer in Tennessee. The calves were trucked to Texas and then treated with nothing or human interferon alpha orally (1 IU/lb) on arrival. The next day during processing the calves were given another 1 IU/lb dose of interferon and were vaccinated as shown in Table 11.

TABLE 11

No. of Calves	Interferon	BVD Vaccine
17	twice	none
17	none	none
17	twice	Diamond Labs (killed)
17	none	Diamond Labs (killed)
17	twice	Nordens MLV TS (Lot A/3.18.85)
17	none	Nordens MLV TS (Lot A/3.18.85)

Tables 12-15 show the results of this test.

TABLE 12

Number of Calves Treated With Antibiotics Because Fever ≥ 104°F

Treatment		No. of Calves Treated On			
BVD Vaccine	Interferon	Arrival	Processing	+1 Day	Later
none	none	2	12	2	0
none	twice	3	8	2	2
killed	none	2	8	1	2
killed	twice	2	8	6	1
live	none	2	10	1	3
live	twice	5	8	1	1

TABLE 13

Morbidity and Retreatment Rates

Treatment		Morbidity	Retreatment
BVD Vaccine	Interferon	Rate(%)	Rate(%)
none	none	93	79
none	twice	86	50
killed	none	73	36
killed	twice	100	53
live	none	83	50
live	twice	93	90

TABLE 14

Fever Duration and Peak Temperature

No. of	Treatment		Fever(Avg.)	
Calves	BVD Vaccine	Interferon	Duration*	Peak(°F)
16	none	none	2.1	105.5
15	none	twice	2.4	105.1
13	killed	none	2.4	105.3
17	killed	twice	1.9	105.2
16	live	none	2.2	105.3
15	live	twice	3.1	105.8

*Duration in days with fever ≥ 104°F.

TABLE 15

Serological Response

No. of Calves	Treatment	No. of Seroconversions	GMT of BVD Antibody 28 Days After Vaccination
15	BVD vaccine + interferon	11	6.65
16	BVD vaccine only	6	3.22

Table 12 shows the number of calves in each group that were treated with antibiotics because of having a fever of at least 104°F. The number is given for the day of arrival, the day of processing, one day after processing, and finally several days after processing. As the "arrival" column shows, substantially more calves in the

group that was vaccinated with live virus and given two doses of interferon were sick before the vaccination. This is probably also reflected to some extent in the results shown in the subsequent tables.

Table 13 shows the percentage morbidity rate, that is, the percentage of calves that required antibiotics treatment, and the percentage of calves that required retreatment with antibiotics. Table 14 shows the fever experienced by the calves in the different groups. In the groups vaccinated with live virus, the duration of fever over 104°F was reduced in calves treated with interferon. Table 15 shows that seroconversion and GMT of BVD antibody were significantly improved in calves treated with interferon.

* * *

If interferon is to be administered to animals simultaneously with the administration of a vaccine, the two can be administered separately or mixed together. ("Simultaneously" is used here and in the claims to mean administration within a few minutes of the same time, not necessarily at the same precise second.) If they are mixed together, the formulation can be the same as standard vaccine formulations (which include a suspension of attenuated or killed microorganisms suitable for inducing immunity to an infectious disease). Such vaccine formulations are well known to those skilled in the art. The only change would be the addition of the necessary amount of a biologically active interferon. Such formulations can include pharmaceutically acceptable carriers such as phosphate buffered saline (PBS).

The preceeding specification describes specific embodiments of this invention for the purposes of illus-

tration and explanation. Those skilled in this art will recognize that many modifications could be made to the materials and methods described that would still be within the scope and spirit of the invention. Applicant intends for the following claims to be interpreted to include all such modifications.

CLAIMS:

1. A method of enhancing the efficiency of a vaccine in warm blooded vertebrates, including administering to a warm blooded vertebrate in conjunction with the administration of a vaccine a biologically active interferon in a dosage no greater than about 5 IU/lb of body weight per day.

2. The method of claim 1 where the dosage is about 1.0 IU/lb of body weight per day.

3. The method of claim 1, where the interferon is administered simultaneously with the vaccine.

4. The method of claim 1, where the interferon is administered within about one day before or after the time when the vaccine is administered.

5. The method of claim 1, where the interferon is administered on two or more of the days in the period consisting of the day before vaccine administration, the day of vaccine administration, and the day after vaccine administration.

6. The method of claim 1, where the interferon is administered orally.

7. The method of claim 1, where the interferon is administered intranasally.

8. The method of claim 1, where the interferon is administered intramuscularly.

9. The method of claim 1, where the interferon is administered intravenously.

10. The method of claim 1, where the interferon is human interferon alpha.

11. The method of claim 1, where the dosage is about 1.0 IU/lb of body weight per day, the interferon is human interferon alpha, and is administered orally simultaneously with the vaccine.

12. A method of enhancing the efficiency of a vaccine in warm blooded vertebrates, including the steps of:

administering a vaccine to a warm blooded vertebrate; and

administering to the warm blooded vertebrate within about one day before or after the administration of the vaccine a biologically active interferon in a dosage no greater than about 5 IU/lb of body weight per day.

13. The method of claim 12, where the interferon is administered simultaneously with the vaccine.

14. A composition for vaccinating warm blooded vertebrates, including:

a suspension of attenuated or killed microorganisms suitable for inducing immunity to an infectious disease; and

a biologically active interferon.

15. The composition of claim 14, also including a pharmaceutically acceptable carrier.

16. The composition of claim 14, where the microorganisms are IBR virus.

17. The composition of claim 14, where the interferon is human interferon alpha.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 86/02783

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [8]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: A 61 K 45/02; A 61 K 39/265

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Biological Abstracts, volume 70, 1980, (Philadelphia, PA., US), I.F. Barinskii et al.: "Comperative studies on the combined use of vaccines, interferon and interferon inducers in some neurovirus infections", see abstract 14841, & Vopr. Virusol. 0(3): 262-267, 1979	14,15
	--	
X	Chemical Abstracts, volume 98, no. 19, 9 May 1983, (Columbus, Ohio, US), I.F. Barinskii et al.: "Combined effect of specific vaccine, exogenous interferon, and interferon inducer on the development of experimental infection with human acute encephalomyelitis virus", see page 367, abstract 158930m, & Vopr. Virusol. 1983, (1), 62-6	14,15
	--	
X	Chemical Abstracts, volume 92, no. 19, 12 May 1980, (Columbus, Ohio, US), T.A. Bektemirov et al.: "Smallpox	
	./.	

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
6th April 1987	12 MAY 1987
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	L. ROSSI

International Application No. PCT/US 86/02783

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

	vaccination under the protection of interferon and its inducer", see page 446, abstract 162033q, & Vopr. Virusol. 1980, (1), 76-8 --	14,15,17
X	Chemical Abstracts, volume 82, no. 23, 9 June 1975, (Columbus, Ohio, US), J.M. Best et al.: "Effect of a human interferon preparation on vaccine-induced rubella infection", see page 399, abstract 153639h, & J. Biol. Stand. 1975, 3(1), 107-12 -- ./..	14,15

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 1-13, because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv)
Methods for treatment of the human or animal body by means of surgery or therapy, as well as diagnostic methods.

2. [ ] Claim numbers ...., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers ...., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

International Application No. PCT/US 86/02783

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	Biological Abstracts, volume 79, 1985, (Philadelphia, PA., US), P.J. Grob et al.: "Interferon as an adjuvant for hepatitis B vaccination in non-responder and low-responder populations", see abstract 13273, & Eur. J. Clin. Microbiol. 3(3): 195-198, 1984	14,15,17
A,P	FR, A, 2575655 (THE TEXAS A & M UNIVERSITY SYSTEM) 11 July 1986 cited in the application	
A	WO, A, 82/00588 (UNIVERSITY OF ILLINOIS FOUNDATION) 4 March 1982 cited in the application	
A	FR, A, 2537436 (THE TEXAS A & M UNIVERSITY SYSTEM) 15 June 1984 cited in the application	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/US 86/02783 (SA 15755)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 14/04/87

The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
FR-A- 2575655	11/07/86	AU-A-	5163085	10/07/86
		DE-A-	3600083	18/09/86
WO-A- 8200588	04/03/82	EP-A-	0058192	25/08/82
		US-A-	4462985	31/07/84
FR-A- 2537436	15/06/84	DE-A-	3345092	14/06/84
		AU-A-	2211283	21/06/84
		US-A-	4497795	05/02/85
		CA-A-	1215645	23/12/86
		AU-B-	558901	12/02/87

For more details about this annex :
see Official Journal of the European Patent Office, No. 12/82